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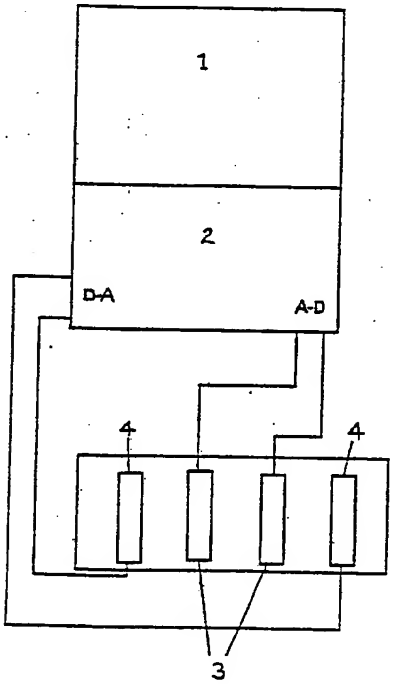

(57) Abstract

Cellular biological material (such as living tissue) is analysed or monitored by applaying an AC electrical potential across the biological material so as to produce a non linear dielectric spectrum, and obtaining a detectable signal corresponding to the resulting spectrum. The potential is of a first frequency and the measured response at one or more second frequency substantially not overlapping with the first frequency.

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Analytical or Monitoring Apparatus and Method

This invention relates to apparatus and method for analysis or monitoring of biological material and particularly, but not exclusively, to apparatus for monitoring or analysis of cellular biological material, in which a response is obtained from enzymes retained within cell membranes.

We have described in our U.K. patent specification 2247530 a method and apparatus for analysis of biological cell material, substrates therefor, or inhibitors of cell metabolism for cell material, the method comprising applying an AC electrical potential across a sample of the biological material so as to produce a non-linear dielectric spectrum, and obtaining a detectable signal corresponding to said spectrum.

The apparatus described in our prior specification, which (as previously indicated) is for analysing biological cell material, substrates therefor, or inhibitors of cell metabolism for cell material, comprises:

(a) retaining means for retaining a sample of biological material;

(b) means for applying an AC electrical potential across the sample so as to produce a non-linear dielectric spectrum; and

(c) means for obtaining a detectable signal corresponding to the spectrum.

According to the present invention, apparatus for monitoring or analysing a determinand associated with cellular biological material, which apparatus comprises:

(a) means for applying an AC electrical potential at one or more discrete frequencies to cellular biological material;

(b) means for determining a response of the material at one or more frequencies which were substantially absent from the applied AC potential; and

(c) means for comparing the response to a stored characteristic of the determinand.

The determinand may be a concentration or other variable in cellular biological material (such as viable or living tissue in, for example, an animal, such as a human animal). An example of a preferred determinand is glucose concentration. The response obtained at one or more frequencies absent from the applied AC potential is referred to as a non-linear dielectric spectrum, as described in more detail in the above U.K. specification.

The apparatus according to the invention can be used, by way of example, in a method of monitoring the ability of living or viable cell material to transduce exogenous electric field energy. We have discovered that the particular harmonics present in the non-linear dielectric spectrum obtained from a cellular biological material are indicative of the metabolic state of living cells in the biological material.

The means for applying an AC potential preferably comprises a plurality of appropriate electrodes, a coil or the like, generally of known type; the electrodes are preferably arranged to be substantially flush with the skin of a patient so that the apparatus can be used for non-invasive monitoring of physiological parameters of the patient.

The means for determining a response of the material at one or more frequencies which were substantially absent from the applied AC potential may be substantially as described in the abovementioned prior specification.

It is therefore not necessary according to the invention to provide a reference non-linear dielectric spectrum; the apparatus may be calibrated by techniques described hereinafter for a first subject, and then used for further subjects of the same general type.

The apparatus according to the invention is preferably provided with means for obtaining a detectable signal, which may, for example, include a chart recorder, screen display, digital display or digital readout.

The mode of operation of apparatus according to the invention, in the method according to the invention, will now be described, by way of illustration, in more detail. When a field of appropriately low frequency is applied to cellular biological material contained between two or more macroscopic electrodes or within a coil, the charging of the membrane capacitance may cause an effective "amplification" of the macroscopic field across the membrane. In certain cases in which the membrane of interest contains appropriate enzymes, this can cause performance of useful biological work in a field- and frequency-dependent fashion. A general mechanism underlying this effect is that enzymes are not dipolar "billiard balls" and can relax between different conformations, some of which may and some of which may not have different vectorial dipole moments from each other.

The electrical potential applied to the biological material may comprise a relatively high field applied to excite the material and a relatively low probing AC voltage to register the field-dependent dielectric properties of the material.

It is preferred to use a sinusoidal AC field to excite the material and the entire frequency range of interest is observed by performing a transformation to see the extent to which the non-linearities of the biological material are manifest by the generation of harmonics. By varying the frequency and amplitude of the exciting current, a multi-dimensional non-linear dielectric spectrum can be built up which can act as a dielectric fingerprint of the sample under test.

Preferably the frequency of the excitation signal is below the range in which β-dispersion of the dielectric permittivity of the test material occurs. Typically therefore the excitation signal frequency is a maximum of 100 kHz (preferably a maximum of 1 kHz). Also, typically the excitation signal has a peak-to-peak value of 20 volts (preferably 4 volts): typically the outer electrodes are 2cm apart giving a field strength of ± 5 volts/cm for a signal of 20 volts peak-to-peak (or $\pm$ 1 volt/cm for a signal of 4 volts peak-to-peak).

Membrane proteins (typically in living tissue) are particularly powerful candidates for interacting with electrical fields for a variety of reasons, including the following: (i) the membrane protein cannot rotate from one side of the membrane to the other and dissipate electrical energy by simple Debye-like rotation of this type; (ii) as described above, the membrane can "amplify" the exciting signal; and (iii) membrane proteins have substantial dipole moments. In addition, of course, in common with all proteins, they can effect transitions between different conformational states possessing different dipole moments. Thus in seeking a mechanistic basis for the remarkable generation of non-linear dielectric spectra that we have observed one is led to consider the membrane properties of cell material present in the biological material under test.

The apparatus is arranged to apply an electrical potential of one or more initial frequencies to the biological material, and to measure the response of the material at at least one response frequency substantially absent from (substantially not overlapping with) the initial frequency or frequencies. In some embodiments, the electrical potential comprises a relatively high field applied to excite the system, and a relatively low probing AC voltage to register the field-dependent dielectric properties of the biological material.

In the apparatus according to the invention, the abovementioned potential is preferably sinusoidal, and the entire frequency range of interest is preferably observed by performing a translation or vector transformation to ascertain the extent to which the non-linearities of the material are manifest by the generation of harmonics.

Other features of the apparatus according to the invention, and its mode of use may be substantially as described in our prior U.K. specification 2247530, as referred to above. The whole of the disclosure thereof is therefore incorporated herein by reference.

Features of the invention will now be described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 shows, schematically, certain features of exemplary apparatus according to the invention; and

Figures 2 to 7 show results achieved in exemplary analyses using method and apparatus according to the invention.

In the embodiment shown in Figure 1, an AC potential of predetermined frequency is applied by generator 2 via digital-to-analogue conversion D-A between an outer pair of electrodes 4 in order to excite the system, and cause an alternating potential to arise between an inner pair of electrodes 3. The AC potential arising between the inner pair of electrodes includes harmonics of the excitation frequency. A computer 1 carries out a Fourier transformation on the signal received from the inner pair of electrodes via analogue-to-digital conversion A-D, to determine the power levels at the first five (for example) harmonics. The process is repeated with different voltages of the excitation signal, and then at different excitation frequencies.

The excitation signal may consist of a sinusoidal waveform. Alternatively the excitation signal may consist of a relatively high DC with a relatively low AC component superimposed on it.

In order that features of the present invention may be more fully understood, the following examples are given by way of illustration only.

In the examples, non-linear dielectric spectroscopy was carried out largely as described in U.K. patent specification 2247530, using in this case a matrix of 5 voltages (zero-to-peak (as measured on the outer electrodes of apparatus as illustrated in Figure 1) and 9 frequencies (in Hz), as follows:

0.500000
0.750000
1.000000 Voltages
1.250000
1.500000

10.000000
17.782794
31.622777
56.234133

100.000000 Frequencies
177.827941
316.227766
562.341325
1000.000000

A sweep consisted of 45 individual spectra, averaging each for 10 blocks. Further sweeps were taken at appropriate intervals. The sampling rate at the inner electrodes was adjusted to be 16 times the value of the frequency applied, such that no windowing was needed and after (Fourier) transformation the power in each consecutive harmonic appears in each consecutive bin. To avoid the need for a reference run (without cells), the following procedure was adopted. The data matrix, consisting of the powers in each of the first 5 harmonics (including the fundamental) at each voltage and frequency, was subjected to multivariate calibration using the partial least squares (PLS) algorithm, fully cross-validated by the leave-one-out method. Such multivariate calibrations are well known to those skilled in the chemometric art.

Example

A spot was marked on a human subject's forearm to ensure repeatable placement of a probe with flush electrodes on subsequent sweeps. The probe was also marked to ensure repeatability of orientation. Before each spectrum was taken, the probe was moistened in 150 mM NaCl to ensure good electrical coupling.

The first two experiments (3 figures) were carried out as follows. Baseline sweeps were taken after the subject had eaten no food for 16 hours, using the same voltages and frequencies as above. Glucose measurements were taken on finger-pricked blood with an optical blood glucose monitoring instrument commercially available under the trade mark "Reflolux" as the reference method. About 100g of glucose were given orally, and further sweeps taken at approx one minute intervals, checking with the Reflolux instrument every 5 minutes and interpolating these reference readings. To improve the ability of the calibration models to generalise, an iterative method for removing outliers was performed, as follows. First the data from a given run (run 1, me8) were used to make the best model,

as judged by cross-validation, leave-one-out self-prediction. The model was used to predict run 2 (me7), and then the points chosen that are closest (within 0.5 mM) to the 1:1 line and the others assumed to be real outliers, i.e. bad data. These "good points" were then used to make a new model, again the best as judged by cross-validated, leave-one-out self-prediction. Finally, a calibration model was formed on the first run with outliers removed according to the revised prediction from the second run. The data for the self model so formed, fully cross validated, using 2 PLS factors, are shown in Figure 2.

Figure 3 shows the predictions from a calibration model of the same data produced on alternate (odd-numbered) points predicting the even-numbered points from the same run.

Figure 4 shows the prediction of the pruned dataset of me7 as predicted from the model formed on the pruned dataset of me8.

Data was acquired from a separate (and diabetic) subject, who had just eaten a meal, his blood glucose followed using the Reflolux instrument and non-linear dielectric spectra acquired exactly as above. The same calibration model as above (formed on the first subject) was used to predict blood glucose data from the second subject (when these were within the range that had been covered by the calibration model), as shown in Figure 5 (in which the dotted lines show accuracies of $\pm$ 10%, the claimed best precision of the reference method).

Finally, a combined model was produced for a separate pair of subjects (one diabetic, one non-diabetic). Figure 6 shows the self-calibration, fully cross-validated, using 5 PLS factors, whilst Figure 7 shows the predictions using data from the same subjects but which had not been included in the calibration model. In each case, the solid line is the line of identity whilst the dotted lines are identity $\pm$ 10%. This shows that the method according to the invention has excellent predictive power.

Claims:

1. Apparatus for monitoring or analysing a determinand associated with cellular biological material, which apparatus comprises:
   (a) means for applying an AC electrical potential at one or more discrete frequencies to said material;
   (b) means for determining a response of said material at one or more frequencies which were substantially absent from the applied AC potential; and
   (c) means for comparing said response to a stored characteristic of said determinand.

2. Apparatus according to claim 1, which further comprises means for retaining said biological material in proximity to said electric potential applying means.

3. Apparatus according to claim 2, wherein said means for retaining the biological material comprises adhesive provided on a patch or the like for retention of an electrode to a subject's skin.

4. Apparatus according to any of claims 1 to 3, wherein the electrical potential comprises a relatively high field to excite the biological material, and a relatively low probing AC voltage to register the field-dependent dielectric properties of the biological material.

5. Apparatus according to any of claims 1 to 4, wherein said potential is sinusoidal, and the apparatus is arranged to scan the entire frequency range of interest by performing a translation or vector transformation to ascertain the extent to which a non-linear dielectric response is manifest by the generation of harmonics.

6. Apparatus according to any of claims 1 to 5, wherein said response comparing means comprises data processing apparatus previously calibrated with a characteristic of cellular biological material of the same general type.

7. Apparatus according to any of claims 1 to 6, which further comprises means for obtaining a detectable signal depending on the results of comparison of said response to said stored characteristic.

8. A method of analysing or monitoring a determinand associated with cellular biological material, which comprises applying an AC electrical potential at one or more discrete initial frequencies to a sample of said material; measuring a response of the material at at least one response frequency substantially not overlapping with said applied AC potential; and comparing said response with a stored characteristic of said determinand.

9. A method according to claim 8, wherein the electrical potential comprises a relatively high field to excite the biological material, and a relatively low probing AC voltage to register the field-dependent dielectric properties of the biological material.

10. A method according to claim 8 or 9, wherein said potential is sinusoidal, and the apparatus is arranged to scan the entire frequency range of interest by performing a translation or vector transformation to ascertain the extent to which a non-linear dielectric response is manifest by the generation of harmonics.

11. A method according to any of claims 8 to 10, wherein said determinand is the concentration of glucose.

FIGURE 1

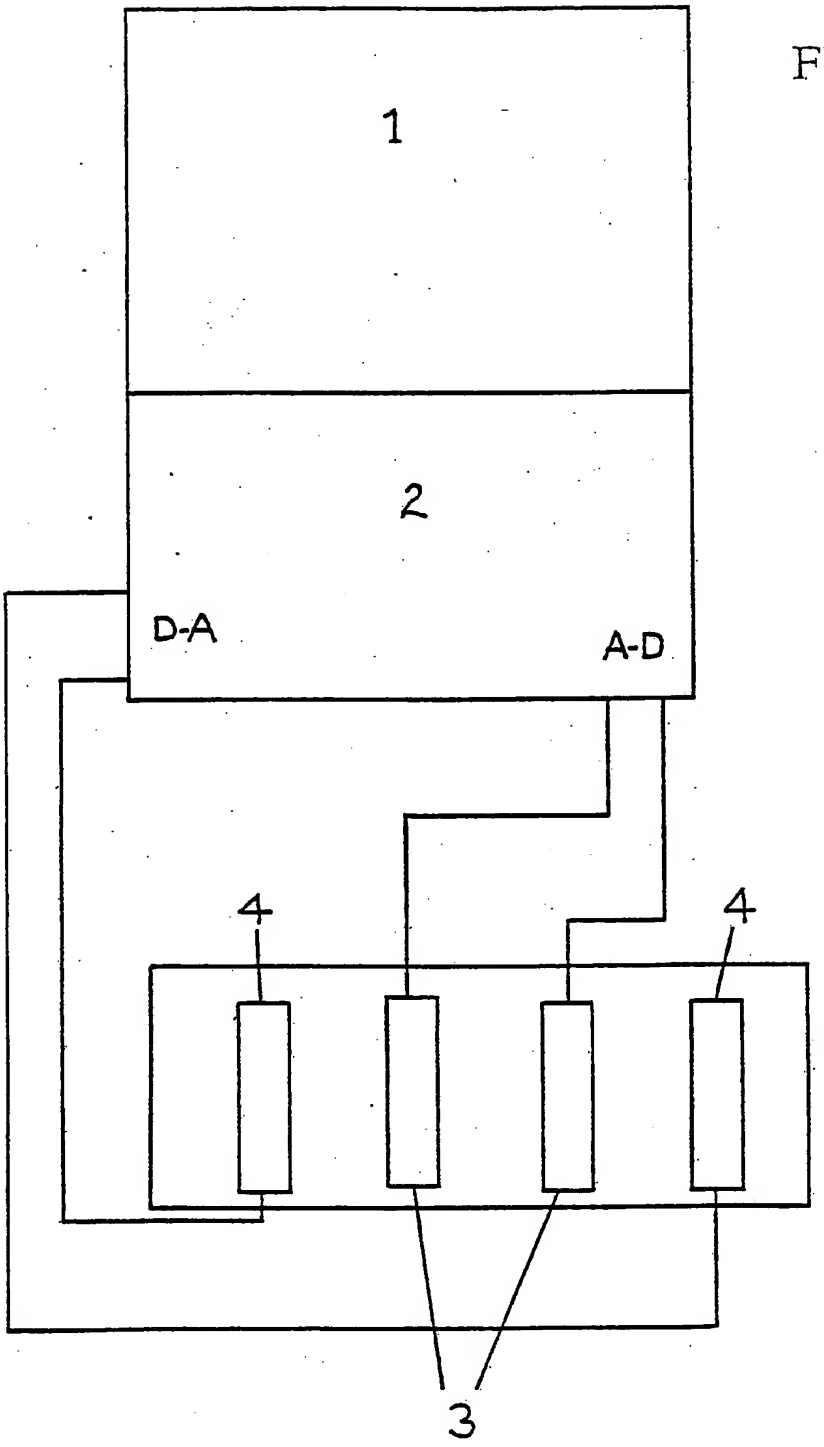
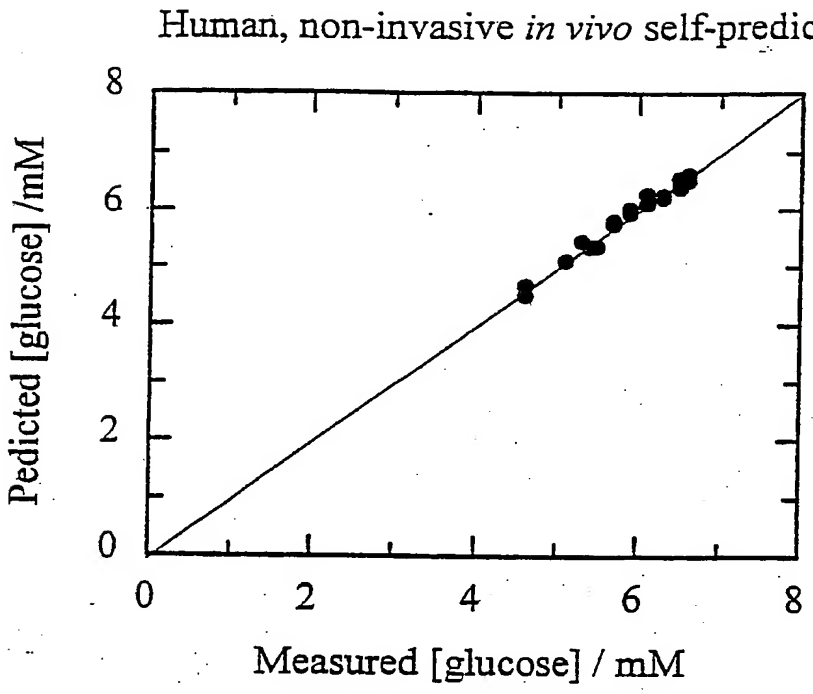


FIGURE 2



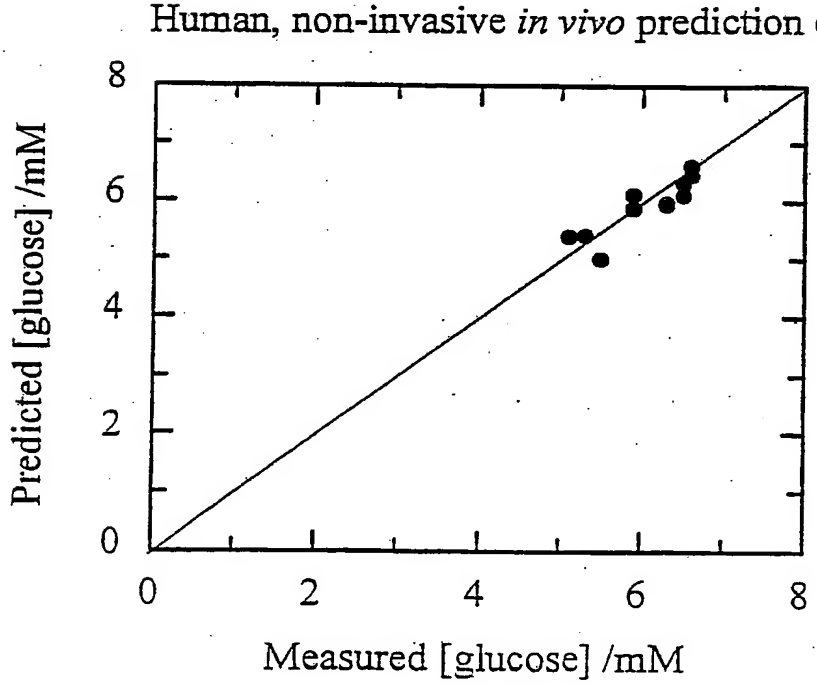


FIGURE 3

Human, non-invasive *in vivo* prediction; model formed on subject 1 predicts data for subject 2

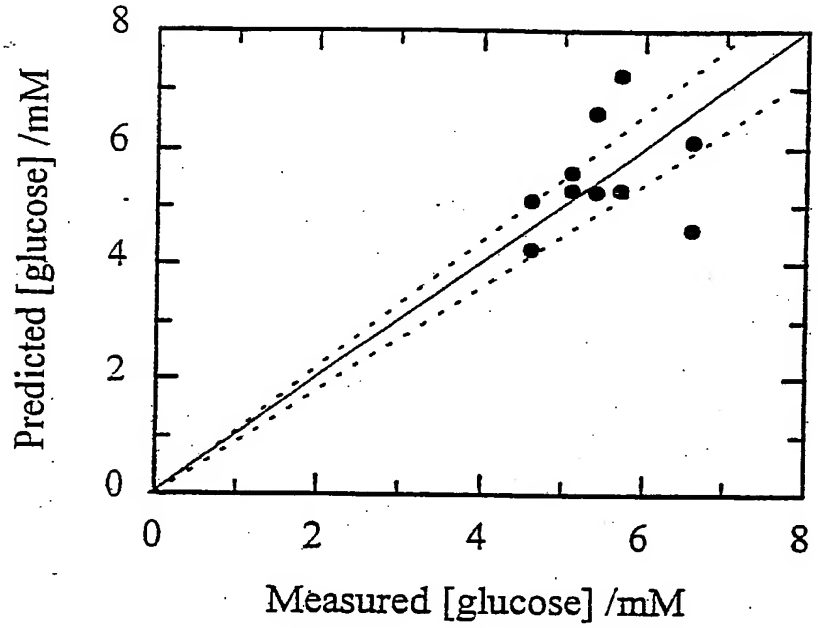


FIGURE 4

Human, non-invasive *in vivo* prediction; run 1 (outliers removed) predicts run 2 (outliers removed)

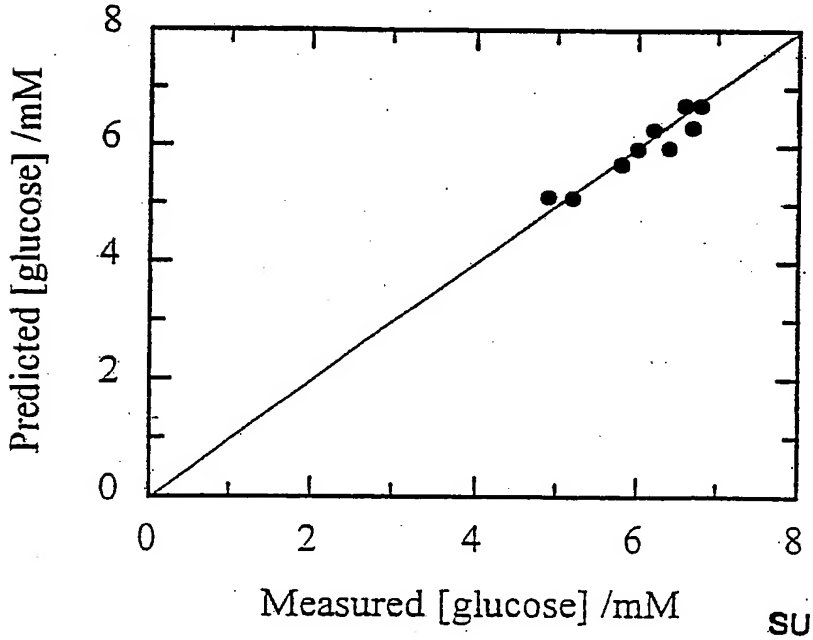


FIGURE 5

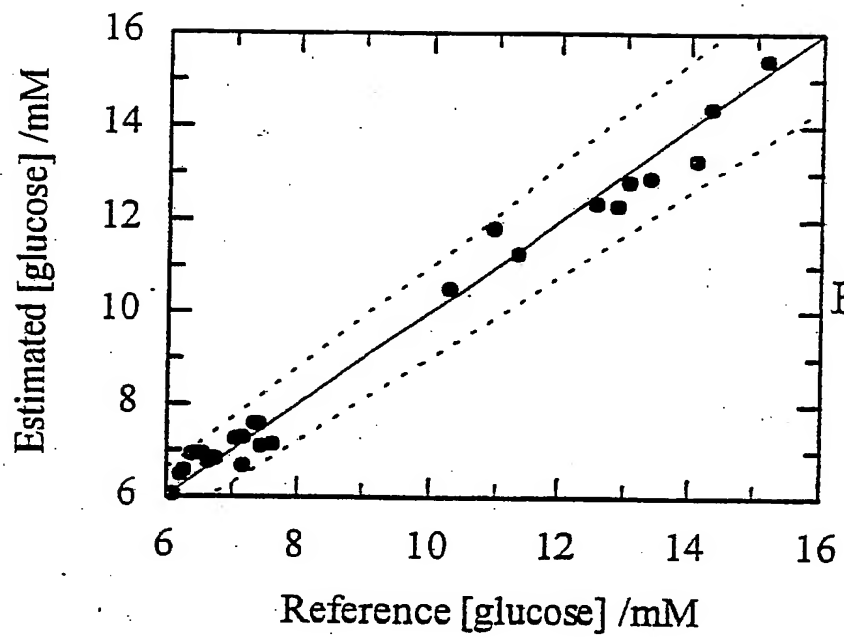


FIGURE 6

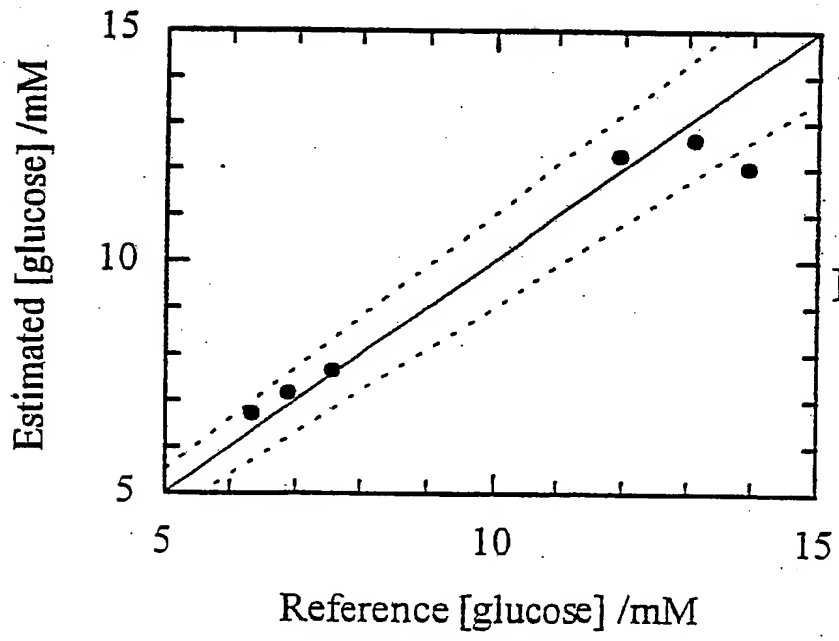


FIGURE 7

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00458

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N33/487; A61B5/05; A61B5/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	G01N ; A61B

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	DE,U,8 903 616 (MEGGL, FRIEDEMANN) 30 August 1990 see the whole document ---	1-11
A	DE,A,3 643 263 (HOMMEL, HORST) 7 July 1988 see the whole document ---	1-11
A	US,A,4 240 027 (LARSEN) 16 December 1980 see column 1, line 15 - column 16, line 30; figures ---	1-11
A	EP,A,0 101 880 (SIEMENS AG) 7 March 1984 see page 1, line 13 - page 12, line 25; figures -----	1-11

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13 JULY 1993	21. 07. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	BOSMA R.A.P.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

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SA 71923

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-U-8903616	19-07-90	None	
DE-A-3643263	07-07-88	None	
US-A-4240027	16-12-80	None	
EP-A-0101880	07-03-84	DE-A- 3228542 US-A- 4919770	02-02-84 24-04-90